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THE EFFECTS OF DIMETHYLSULFOXIDE, DIMETHYLFORMAMIDE AND DIMETHYLACETAMIDE ON THE CUTANEOUS BARRIER TO WATER IN HUMAN SKIN*

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The stratum corneum is the principal barrier to the movement of water and other substances across the epidermis (1). This barrier is not absolute and under normal conditions small but measurable amounts of water are lost from the body by transepidermal diffusion (2, 3). The magnitude of this loss at any given site depends upon a number of factors, principally the environmental humidity and temperature (4), skin surface temperature (1) and stratum corneum thickness (5). There is general agreement that under experimental conditions of zero external humidity, diffusive loss is about 0.20 to 0.30 mg cm^{-2} hr^{-1} for most central areas of the body (6).

When the stratum corneum is damaged or diseased, diffusive water loss is increased. This has been amply demonstrated in experimental injury to the stratum corneum by cellophane tape stripping (7) and in disease characterized by the formation of a defective horny layer (8). In these conditions percutaneous absorption of a variety of substances is also increased (9). Although the cutaneous permeability characteristics for no two substances are exactly the same (10), the measurement of outward transepidermal water diffusion provides one useful parameter of the barrier function of the stratum corneum. The precise way in which the stratum corneum acts as a barrier to diffusion of all molecules is not understood and the relative importance of intracellular cements, cell membranes, keratin fibers and other cell constituents is controversial (10, 11).

The recent observation that certain organic solvents such as dimethylsulfoxide (DMSO) may profoundly affect the function of the stratum corneum is therefore of considerable importance, theoretically for the light it may throw on barrier mechanisms and practically in that these compounds may have a future place in topical therapy. Incorporation of a variety of pharmacologically active agents in DMSO greatly enhances their percutaneous absorption (12). In addition these solvents facilitate the establishment of a reservoir of the penetrant in the stratum corneum (13).

It has thus become clear that DMSO and the related compounds dimethylacetamide (DMA) and dimethylformamide (DMF) (14) share the remarkable property of being able to suppress barrier function but the extent to which they can achieve this, the duration of the effect and its degree of reversibility *in vivo* have not been established.

The purpose of the experiments described below has been to measure the degree and duration of barrier suppression induced by DMSO, DMA and DMF. This has been done *in vivo* in normal human skin using outward transepidermal water diffusion as the parameter of barrier function.

METHODS

Transepidermal water loss was measured by electro-hygrometry using a modification of a method previously described (6). Dry nitrogen was passed through a skin chamber strapped to the flexor aspect of the mid-forearm, the temperature and relative humidity of the gas stream being measured proximal and distal to the skin chamber by means of interposed sensors. If the rate of gas flow and skin surface area exposed are known, the water loss from the skin surface can be calculated (15).

Anhidrosis was induced in the test area by the topical application of poldine methosulphate (4% in water) under overnight occlusion (16). Complete sweat suppression was checked by direct visualization of sweat pores using the iodine and starch-oil method during thermal stress induced by exercise, exposure to a radiant heat cradle or both. Complete absence of a sweat pore pattern in the test area in the presence of evidence of brisk sweating in the entire surrounding area was accepted as evidence of anhidrosis and confirmed by the subsequent failure of thermal stimulation to alter the basal hygrometric readings. We confirmed a previous report of the efficiency of poldine as a sweat suppressant (16).

Two hours were allowed to elapse after removal

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of the occlusive poldine dressings before anhidrosis was tested to ensure complete desorption of the occlusively hydrated horny layer and a further hour after the sweat test before the basal hygrometric reading was taken. As soon as the latter had been completed 2 ml of solvent were applied to the 4 cm x 4 cm test area on a piece of lint which was left *in situ* for 30 minutes. At the end of this period the lint was removed, the skin wiped dry and exposed to room air for 30 minutes and to a stream of dry nitrogen for a further 30 minutes to ensure that no occlusive effect induced by the application interfered with subsequent measurements.

Water loss from the test area was measured 1½, 3, 4½, 6½ and 24 hours after the onset of exposure to the solvent. In some experiments readings were taken at other times. Poldine was reapplied between the 6½ and 24 hour measurements to ensure continued anhidrosis. The effects of undiluted DMSO, DMA and DMF, each applied as above for 30 minutes, were each studied in 3 subjects. Different subjects were used and the effects of the solvents were not compared in the same persons. Three other subjects had further studies with DMSO alone under varied conditions. In two, smaller amounts of DMSO were applied and left on for 15 minutes only. In the third, water loss was first measured 38 minutes after first exposure and 13 minutes after removal of the DMSO from the skin surface.

RESULTS

The effects of pure DMSO, DMF and DMA are shown separately in Figs. 1, 2 and 3 and are compared in Fig. 4.

Dimethylsulfoxide (DMSO). Exposure of skin to pure DMSO for 30 minutes without occlusion had a profound effect on water loss. Ninety minutes after first exposure the loss was increased 8-, 11- and 17-fold respectively in 3 subjects (Fig. 1) but the effect was rapidly reversed. Within 6½ hours loss was reduced to between 3- and 4-fold the basal levels. Thereafter, restoration of barrier function was much slower and the latter did not return completely to normal. Pure DMSO is an irritant and after 2 to 3 days all 3 subjects showed some scaling indicating accelerated epidermal cell replacement and the formation of a defective horny layer. This was associated with

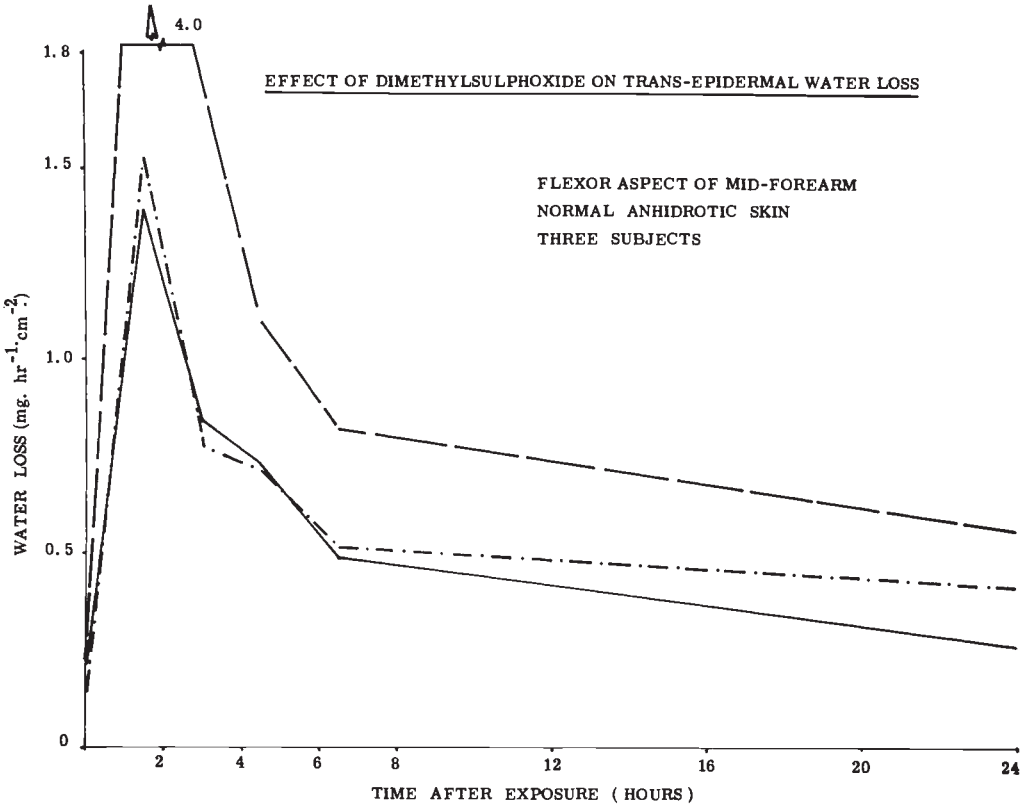

FIG. 1. Effect of Dimethylsulfoxide on Transepidermal Water Loss

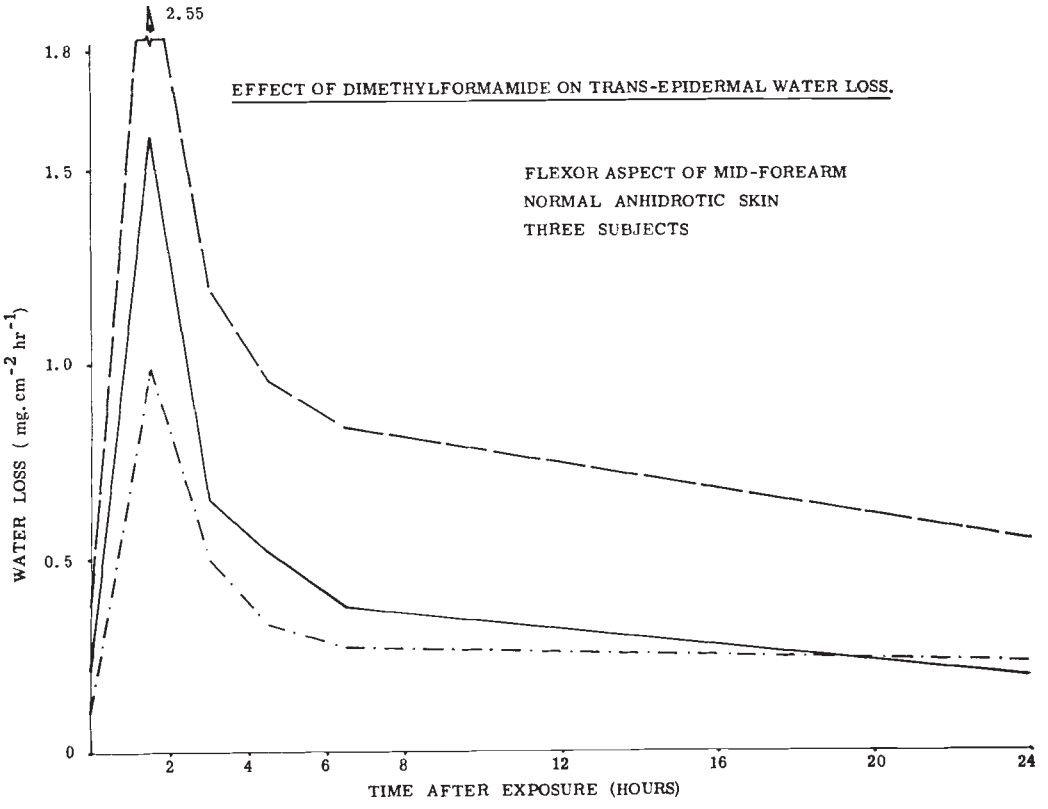

FIG. 2. Effect of Dimethylformamide on Transepidermal Water Loss

a secondary rise in water loss which only returned to normal slowly over the following 2 weeks.

In other experiments, 2 ml DMSO were painted on to the forearm of 2 other subjects and left for 15 minutes before being wiped away. The diffusive water loss was approximately doubled and trebled respectively in the two subjects in comparison with their basal readings.

In the last experiment with DMSO 5 ml were applied on lint for 15 minutes only, the skin wiped dry and dry air blown over the site for 10 minutes before passing the gas flow through the sensors. Compared with a pre-treatment reading of 0.29 mg/hr^{-1}/cm^{-2}. the loss was 3.35 mg/hr^{-1}/cm^{-2}. 13 minutes after removal of the dressing and 38 minutes after first application of the DMSO.

DMSO, applied in this manner, constantly induced marked erythema and whealing lasting 3–6 hours.

Dimethylformamide (*DMF*). The pattern of change in barrier function induced by application of pure DMF under identical conditions was similar but the rise in water loss was less dramatic and subsequent drop more rapid and complete (Fig. 2). In 2 of the 3 subjects barrier function was virtually completely restored in 24 hours. DMF constantly caused less erythema and whealing than DMSO, lasting about 1 to 3 hours.

Dimethylacetamide (*DMA*). DMA had a much smaller effect on water loss which was remarkably constant in the 3 subjects studied. A peak 3-fold effect was shown by all the subjects. Subsequently the loss rapidly dropped, was almost normal again at 6½ hours and virtually completely normal at 24 hours (Fig. 3). Only slight erythema in 2 of the 3 subjects was produced by the application.

DISCUSSION

The three substances studied here are assuming an increasing importance as solvents and reactants in industry. DMSO has been

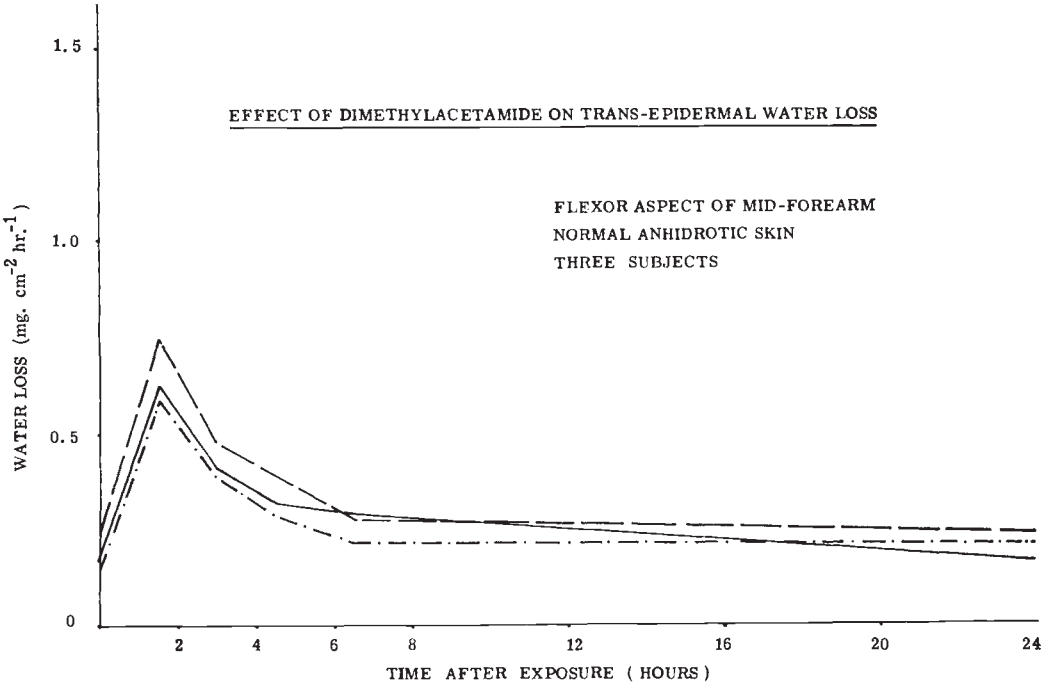

FIG. 3. Effect of Dimethylacetamide on Transepidermal Water Loss

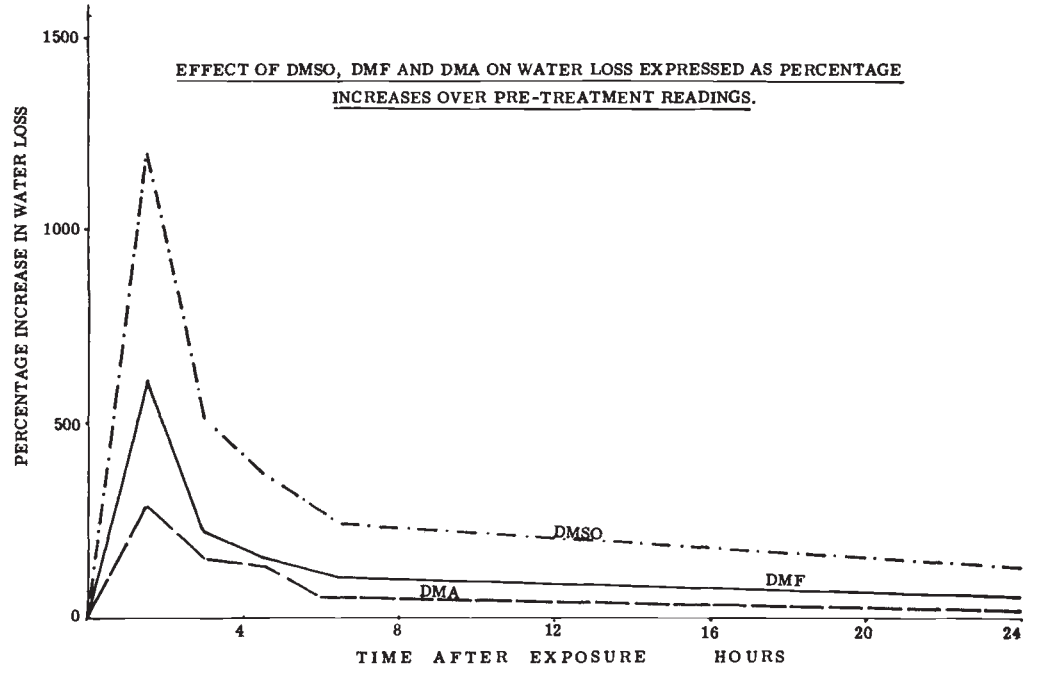

FIG. 4. Effect of DMSO, DMF and DMA on Water Loss Expressed as Percentage Increases Over Pre-treatment Readings.

found to be an effective agent for protection of mammalian cells against freezing (17), apparently being generally non-damaging to cells and tissues (18). It enhances absorption of a wide variety of substances through different biological membranes (19, 20).

The effects of DMSO on water movement through mouse skin have been studied *in vitro* (21). Sweeny and colleagues drew 3 conclusions from these studies, namely, that concentration of DMSO applied was more important than duration of exposure, that the effects of high concentrations of DMSO were irreversible *in vitro* and that DMSO did not appear to have a special ability to promote passage of water through skin by its mere presence. Preliminary observations on human skin *in vivo* led us to doubt the validity of one of these conclusions (6) concerning the irreversibility of the changes induced.

The data presented above indicate clearly that DMSO, DMF and DMA exert a profound but temporary effect on the cutaneous barrier. The speed with which barrier suppression is produced implies a direct effect of the solvent on the stratum corneum. Inflammation in the underlying skin may occur in some subjects as the result of the application of the pure solvents (particularly DMSO) as shown by erythema and whealing, but it has previously been shown that the production of erythema, pallor or whealing by substances which do not directly affect the stratum corneum does not materially influence the rate of water diffusion through anhidrotic skin. Naphazoline hydrochloride (Privine®) (6) or corticosteroid (22, 23) induced pallor and tetrahydrofurfuryl nicotinate (Trafuril®) induced erythema and whealing (6, 22) produce only minor disturbances of transepidermal water diffusion. Inflammation may accelerate epidermal cell replacement with the subsequent development of a parakeratotic scaly horny layer but these changes cannot occur quickly enough to account for the results reported here.

In most of the experiments ample time (1 hour) was allowed after removal of the solvent from the skin surface before water loss was measured. This delay was to ensure that any slight occlusive effect of the application was reversed. In the one experiment where the loss was measured 13 minutes after removal of the dressing it proved to be greatly increased. There may well be considerable barrier impairment within 2 to 3 minutes of application of DMSO and DMF to the skin.

The mode of action of these substances is not understood. Profound electron microscopic changes in the horny layer of the guinea pig produced by DMSO have been demonstrated (24). It is certainly not due simply to a removal of surface lipid or superficial horn fat. Other powerful lipid solvents such as ether, acetone and chloroform:methanol have virtually no effect, however applied, on human transepidermal water loss *in vivo* (6) although the barrier to water can be destroyed in vitro by boiling isolated stratum corneum membranes in such solvents (25). DMSO, DMF and DMA are all strongly hygroscopic and it is likely that the presence of these substances in the stratum corneum greatly increases the hydration of the tissue and therefore its permeability. Two factors, underlying dermal inflammation and heat produced by the interaction of the solvent and water within the stratum corneum, would also lead to a small rise in the temperature and therefore the permeability of the horny layer (26). Neither of these factors could conceivably explain the magnitude of the increased diffusion. There is ample evidence of the ability of water (21) and many other substances to move in an inward direction through the skin under the influence of DMSO (20, 12).

Whatever the mechanism involved, its reversibility is remarkable. It is tempting to speculate that this effect lasts while the solvent is held in the stratum corneum and that as it is absorbed or washed out barrier function returns.

It has been suggested before that substances of this type might have therapeutic usefulness as topical vehicles for other active agents. This approach might allow a high local concentration of a drug to be brought to bear on localized dermal or subcutaneous disease where such a concentration would be too toxic to tissues elsewhere.

The ideal barrier suppressant for this purpose should have the following properties:

1. High degree of barrier suppression.
2. Short duration of suppression.

3. Complete return of barrier function to normal.
4. Non-toxic and non-allergenic if absorbed.
5. Non-irritant to skin.
6. Wide solvent powers.

Of the 3 substances studied here none approaches this perfection. Fig. 4 suggests that of the 3 substances studied here DMF comes nearest to this ideal. DMSO has a greater suppressive effect on barrier function but this is only partially reversed; DMA has a much weaker effect. DMF on the other hand, under the conditions of these experiments, combined marked barrier suppression with rapid return to near normal function.

SUMMARY

Transepidermal water diffusion has been measured as a parameter of stratum corneum barrier function. The method has been used to study the functional effects of dimethylsulfoxide (DMSO), dimethylformamide (DMF) and dimethylacetamide (DMA) applied topically to the stratum corneum of normal skin *in vivo*. The results indicate that these substances all have the ability to produce a striking but reversible suppression of barrier resistance. The suppression was greatest with DMSO and least with DMA. Restoration of normal function was fastest with DMA and slowest with DMSO. DMF produced high barrier suppression with rapid return to near normal. The theoretical and therapeutic implications of these findings have been discussed.

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